V2/V3 Protein Additional Indexes Guide

If more than 8 protein samples need to be multiplexed on *one lane on an Illumina sequencer*, the following **additional i5 and i7 indexes** may be purchased from IDT and used for Library PCR by making unique pairs following the instructions below.

i5 index Primer	Sequence	Index Sequence
i5_Unique_9	AATGATACGGCGACCACCGAGATCTACAC AGAACGAG TCGTCGGCAGCGTC	AGAACGAG
i5_Unique_10	AATGATACGGCGACCACCGAGATCTACAC TGCTTCCA TCGTCGGCAGCGTC	TGCTTCCA
i5_Unique_11	AATGATACGGCGACCACCGAGATCTACAC CTTCGACT TCGTCGGCAGCGTC	CTTCGACT
i5_Unique_12	AATGATACGGCGACCACCGAGATCTACAC CACCTGTT TCGTCGGCAGCGTC	CACCTGTT
i5_Unique_13	AATGATACGGCGACCACCGAGATCTACAC ATCACACG TCGTCGGCAGCGTC	ATCACACG
i5_Unique_14	AATGATACGGCGACCACCGAGATCTACAC CCGTAAGA TCGTCGGCAGCGTC	CCGTAAGA
i5_Unique_15	AATGATACGGCGACCACCGAGATCTACAC TACGCCTT TCGTCGGCAGCGTC	TACGCCTT
i5_Unique_16	AATGATACGGCGACCACCGAGATCTACAC CGACGTTA TCGTCGGCAGCGTC	CGACGTTA

Protein_i7 index Primer	Sequence	Index Sequence
Protein_i7_Unique_9	CAAGCAGAAGACGGCATACGAGAT AGCGTAGC GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGAGATGACTACGCTACTCATGG	AGCGTAGC
Protein_i7_Unique_10	CAAGCAGAAGACGGCATACGAGAT CAGCCTCG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGAGATGACTACGCTACTCATGG	CAGCCTCG
Protein_i7_Unique_11	CAAGCAGAAGACGGCATACGAGAT TGCCTCTT GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGAGATGACTACGCTACTCATGG	TGCCTCTT
Protein_i7_Unique_12	CAAGCAGAAGACGGCATACGAGAT TCCTCTAC GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGAGATGACTACGCTACTCATGG	TCCTCTAC
Protein_i7_Unique_13	CAAGCAGAAGACGGCATACGAGAT TCATGAGC GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGAGATGACTACGCTACTCATGG	TCATGAGC
Protein_i7_Unique_14	CAAGCAGAAGACGGCATACGAGAT CCTGAGAT GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGAGATGACTACGCTACTCATGG	CCTGAGAT
Protein_i7_Unique_15	CAAGCAGAAGACGGCATACGAGAT TAGCGAGT GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGAGATGACTACGCTACTCATGG	TAGCGAGT
Protein_i7_Unique_16	CAAGCAGAAGACGGCATACGAGAT GTAGCTCC GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGAGATGACTACGCTACTCATGG	GTAGCTCC

ORDER

1. Order oligos (single-stranded DNA) on IDT: https://www.idtdna.com/pages/support/how-to-order
2. Purification method: **standard desalting, 4 nmole Ultramer DNA oligo**

LIBRARY PCR SETUP

3. Dilute each oligo to 4 µM with nuclease-free water (typically from 100 µM stock)
4. For each sample combine **5 µl of i5 index primer** and **5 µl of i7 index primer** → The combined primers represent the “Protein Index Primer”
5. Proceed as outlined in the User Guide